

and decreases, and are capable of flowing in less than 30 s when the vessel is turned over.

5 The polymer T16, on the other hand, gives rise to a gel-type state at 60°C for concentrations of 8 g/100 ml and more.

#### EXAMPLES 4:

10 Properties of separation of separation media for capillary electrophoresis comprising, as copolymer, one of the copolymers prepared according to Example 2.

15 The electrophoresis experiments presented in this example and in the following examples were carried out using a laboratory-constructed apparatus similar to that described in Lindberg et al., Electrophoresis, 18, 1973 (1997). The DNAs separated are detected by fluorescence with excitation by an Argon laser at 488 nm and emission at 530 +/- 30 nm. The injection is  
20 of the electrokinetic type. The capillary, made from molten silica coated with polyimide (polymicro), having a diameter of less than 100 µm, is thermostated between the point of injection and the point of detection by circulation of silicone oil in a sealed envelope, with  
25 the exception of the first 2 and last 2 centimetres (unless otherwise stated, this type of capillary will be used in the electrophoresis experiments presented below).

#### 30 Trial 4-1

Separating properties of a medium comprising, as copolymer, the copolymer PAM-NIPAM (T15) at a concentration of 2 g/100 ml.

35 The medium is mixed with TRIS-TAPS buffer (50 mM) and DNA marker (for SYBR GREEN I 10<sup>-4</sup>).

In this particular case, the filling of the capillary is carried out at 25°C, the injection is carried out

over 10 seconds at 25 volts per centimetre and the sample to be separated is of the same nature as that of the preceding trial.

- 5 The separating properties of the said medium are evaluated at two temperatures, 25°C and 60°C, and Figures 3a and 3b present these properties. It is observed that the separation has a higher resolution and is more rapid at 60°C.

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Trial 4-2

Separating properties of a separation medium based on the copolymer PAM-NIPAM (T12).

- 15 The medium is introduced into the capillary at 25°C at a concentration of 8 g/100 ml, mixed with TRIS-TAPS buffer (50 mM) and with the DNA marker SYBR GREEN I (molecular probes) diluted at the rate of  $10^{-4}$  relative to the stock solution sold by the supplier. A similar  
20 behaviour is noted with an improvement in resolution and in separating time when the temperature chosen for the separation is 60°C.

- It is also observed that the resolution for large  
25 duplex DNA fragments is better with the copolymer T15, prepared with a low level of transfer agent and therefore exhibiting a high molecular mass, than with the copolymer T12 prepared with a higher level of transfer agent and exhibiting a lower molecular mass.

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Trial 4-3

Separating properties of a medium comprising, as copolymer, the copolymer PDMAM-NIPAM (T7) at a concentration of 2 g/100 ml.

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This medium is mixed with the TRIS-acetate buffer (50 mM) and with the DNA marker SYBR GREEN I  $10^{-4}$ .

In this particular case, the sample is the marker "Phi-X 174-RF DNA, Hae III digest" (Pharmacia biotech), whose fragments have sizes of between 72 and 1358 bp, the injection is carried out over 5 seconds at 20 volts per centimetre and the sample to be separated is of the same nature as that in the preceding trial.

The separating properties of the said medium are evaluated at two temperatures, 25°C and 50°C, and Figures 4a and 4b present these properties. It is observed, as in the preceding trial, that the separation has a higher resolution and is more rapid at a higher temperature.

Trial 4-4

Separating properties of a medium comprising, as copolymer, the copolymer PAM-NIPAM T21, prepared from the macromonomer PNIPAM-20, at a concentration of 2 g/100 ml.

The medium is mixed with the TRIS-acetate buffer (50 mM). For this separation medium, the viscosity at high temperature is relatively low, and does not allow total suppression of electroosmosis. Consequently, the capillary was, prior to its use, washed with a 1M hydrochloric acid solution comprising 1 g/100 ml of polyvinylpyrrolidone, having a molecular mass of 1 000 000 (Polysciences, Eppelheim, D).

In this particular case, the sample is the marker "100 bp fluorescein ladder", Bio-Rad, whose fragments have sizes of between 100 and 1000 bp, the injection is carried out over 10 seconds at 25 volts per centimetre.

The separating properties of the said medium, introduced into the capillary at 25°C, are evaluated at two temperatures, 25°C and 50°C, and Figures 5a and 5b present these properties.